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Effects of irrigation over three years on the amino acid composition of Treixadura (*Vitis vinifera* L.) musts and wines, and on the aromatic composition and sensory profiles of its wines

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ABSTRACT

Amino acids and volatile compounds play an important role in wine aroma and sensory characteristics. The concentrations of these compounds might be altered by climate interanual variability and by management practices such as irrigation. The aim of the current study was to assess the amino acid profile of musts and wines, volatile composition and sensory profile of wines from *Vitis vinifera* (L.) cultivar 'Treixadura' obtained from vines under rain-fed and irrigation conditions over three consecutive vintages (2012–2014). Musts and wines from the irrigation treatment tended to be higher in acidity than those from rain-fed. However, amino acid and aromatic profiles were mostly affected by climate conditions of each year; although irrigation exerted a significant effect on several compounds (proline, cysteine, tryptophan, phenylalanine, α-terpineol and geraniol). Wines from both treatments received similar marks in the sensory tests, suggesting that irrigation did not greatly modify wine quality under the conditions of this study.

1. Introduction

Amino acids in musts play a role of paramount importance during alcoholic fermentation (Bell & Henschke, 2005). They constitute a nitrogen source for yeasts and their concentration depends on a great number of factors including grapevine variety, fertilization, soil and climate conditions, vineyard management and enological practices (Garde Cerdán et al., 2014). Moreover, the amino acid profile of the musts will influence final wine quality due to the transformations that amino acids undergo during the fermentation process, producing volatile compounds such as higher alcohols, volatile fatty acids and ethyl esters (Swiegers, Bartowsky, Henschke, & Pretorius, 2005) that define wine aroma.

Higher alcohols are the most abundant volatile compounds produced during alcoholic fermentation. When the total concentration of these compounds is below 300 mg L^{-1} they intensify floral notes in wines, whereas they are negative to wine quality when their concentration surpasses that threshold (Hirst & Ritcher, 2016). Ethyl esters are responsible for floral and fruity nuances in young wines (Falqué, Darriet, Fernández, & Dubourdieu, 2008); whereas volatile fatty acids might be negative to wine quality when they are found over their perception threshold, providing cheesy and buttery notes to wines.

Volatile compounds accumulate in grapes during ripening depending on temperature and water availability (Robinson et al., 2014). Therefore, irrigation management in the vineyard is a fundamental tool for controlling berry growth and quality (Jackson & Lombard, 1993). In fact, concerns about climate change altering the temporal distribution of rainfall and increasing the drought events have raised a great concern in viticultural regions worldwide (Fraga, Malheiro, Moutinho-Pereira, & Santos, 2013) and irrigation is increasingly being used to cope with this and to minimize interannual variability in yields even in cool humid regions such as Galicia, northwest Spain (Cancela et al., 2016). Nevertheless, the effects that irrigation practices might have on berry composition and wine aromatic profile have seldom been studied,

Abbreviations: ALB, Albariño; AOC, Appellation d'Origine Controllée; DEEMM, diethyl ethoxymethylenemalonate; AOC, Appellation of Origin; EFC, electronic flow control; ET_c, crop evapotranspiration; ET_0, potential evapotranspiration; GC, Gas Chromatography; HPLC, High-Performance Liquid Chromatography; IS, Internal Standard; LOD, Limit of Detection; LOQ, Limit of Quantification; MS, mass spectrophotometer; OAV, Odour Activity Value; PCA, Principal Component Analysis; PC, Principal Component; UV, Ultraviolet. Amino acids; Arg, arginine; GABA, aminobutyric acid; Thr, threonine; Ala, alanine; Ser, serine; Glu, glutamine; Glu acid, glutamic acid; Val, valine; Phe, phenylalanine; Leu, leucine; His, histidine; Asp acid, aspartic acid; Ile, isoleucine; Try, tryptophan; Tyr, tyrosine; Asp, asparagine; Lys, lysine; Cys, cysteine; Pro, proline; Gly, glycine; Orn, ornithine; Met, methionine

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and contrasting results have emerged, depending on the cultivar and colour of the grapes (Deluc et al., 2009) and on the intensity of water stress, which is usually low in cool-humid areas (Balint & Reynolds, 2017). In this sense, Matthews, Ishii, Anderson, and O'Mahony (1990) concluded that irrigation can modulate wine sensory characteristics, but Talaverano et al. (2017) observed that irrigation only exerted a significant influence on alcohols and C6 compounds. Studies on white varieties are scarcer; Ortega Heras et al. (2014) reported that irrigation increased the amino acid concentrations in berries of the cultivar Verdejo, which could have altered the wine aromatic composition. Furthermore, water stress affected amino acids metabolism of the red cultivar Cabernet Sauvignon but not that of the white cultivar Chardonnay (Deluc et al., 2009); basically, proline concentrations increased with water deficit.

In the Northwest of the Iberian Peninsula (Galicia and North of Portugal), white grapevine cultivars are predominantly grown and their organoleptic characteristics might be altered by increasing temperatures and the occurrence of drought periods during the growing cycle. Among the cultivars that are traditionally grown in these regions, Treixadura is one of the most important since it is used to obtain balanced wines with a high aromatic potential (Falqué, Fernández, & Dubourdieu, 2002). Volatile composition of wines produced with this cultivar has already been described (Blanco, Mirás-Avalos, Suárez, & Orriols, 2012), but the amino acid profile of the musts and wines, as well as the effect that irrigation exerts on them has not been previously assessed. Therefore, the aim of the current study was to assess the effects of irrigation on the amino acid composition of musts and wines, and the aromatic and sensory profiles of wines from the white grapevine (*Vitis vinifera* L.) cultivar Treixadura, traditionally grown in Galicia, during three consecutive years (2012, 2013 and 2014) in Ribeiro.

2. Materials and methods

2.1. Chemical reagents

A Milli-Q equipment (Millipore, Bedford, MA, USA) was used for generating ultra-pure water. Super-gradient HPLC grade acetonitrile and methanol were purchased from Scharlau (Sentmenat, Spain). Ammonium chloride was from Merck (Darmstadt, Germany). L-α amino acids: aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, threonine, arginine, alanine, γ-aminobutyric acid (GABA), proline, tyrosine, valine, methionine, cysteine, isoleucine, tryptophan, leucine, phenylalanine, ornithine hydrochloride, lysine, 2-aminoadipic acid and diethylethoxymethylenemalonate (DEEMM) were from Acros Organics (New Jersey, USA). Solutions of amino acids were prepared with HCl 0.1N.

For the extraction of free terpenes, volatile fatty acids, ethyl esters, acetates of higher alcohols and C6 alcohols, we used dichloromethane, *n*-pentane and anhydrous sodium sulfate (Scharlau, Sentmenat, Spain). Standards for volatile compounds were purchased from different suppliers: Merck (Madrid, Spain), Aldrich (Madrid, Spain), Fluka (Seelze, Germany), Alfa Aesar (Barcelona, Spain) and Sigma (Madrid, Spain). The internal standards (Merck, Madrid, Spain) used for each method were 4-methyl-2-pentanol in the case of major volatile compounds (methanol, higher alcohols, acetaldehyde, ethyl acetate, ethyl lactate, acetoine, 2-phenyl-ethanol and 2,3-butanediol); 4-decanol for terpenes and C6 alcohols; 1-heptanol for volatile fatty acids, ethyl esters and acetates of higher alcohols. All the standards were prepared in 50% hydroalcoholic solutions.

2.2. Study site and experimental design

The experiment was conducted over three consecutive growing seasons (2012, 2013, and 2014) in a 0.2 ha Treixadura vineyard, located in the experimental farm of the Estación de Viticultura e Enoloxía de Galicia (EVEGA), in Leiro (42°21.6′ N, 8°7.02′ W, elevation 115 m), Ourense, Spain, within the Appellation d'Origine Controllée (AOC) Ribeiro (Mirás-Avalos, Trigo-Córdoba, Bouzas-Cid, & Orriols-Fernández, 2016).

Treixadura vines, grafted in 1998 on 196-17C rootstock, were vertically trellised on a single cordon system (10–12 buds per vine). Rows were East-West oriented; spacings were 1.25 and 2.4 m between vines and rows, respectively. Soil was sandy textured, slightly acidic, and of medium fertility. Treatments consisted of a rain-fed and an irrigation to the 50% of crop evapotranspiration (ET_c), using a crop coefficient equal to 0.8 taken from previous reports on vineyards with developed canopies (Fandiño et al., 2012; Williams, 2012). Each treatment had three replicates (three rows of 8 vines each) in a randomized complete block design. Water was applied with two pressure-compensated emitters of 4 L h^{-1} located 25 cm on either side of the vine. Irrigation was applied, according to the former week ET_c from end June to mid-August, approximately two weeks before harvest. Total water amounts applied were 50, 79 and 50 mm for 2012, 2013 and 2014, respectively (Trigo Córdoba, Bouzas Cid, Orriols Fernández, & Mirás Avalos, 2015), allowing for a clear differentiation on grapevine water status under both treatments. Rainfall over the growing season (April to harvest) was 313, 163 and 185 mm for 2012, 2013 and 2014, respectively. Moreover, the mean temperature for the growing season increased from year to year (Supplementary Table 1).

Physiological and production data have been reported elsewhere (Trigo Córdoba et al., 2015). In summary, rain-fed vines showed more negative midday stem water potentials than those irrigated during the three studied years; whereas yield was significantly higher in irrigated vines only in 2014 and this was caused by a greater number of clusters per vine (Supplementary Table 2).

2.3. Samples

Grapes from the different treatments were manually harvested on the same day and transported to the experimental winery. Vinifications were performed separately on samples of about 40 kg per replicate, thus three vinifications per treatment were carried out each year, as previously described for another white variety (Mirás-Avalos et al., 2016).

Briefly, grapes from each replicate were separately destemmed, crushed and pressed in a pneumatic press (yielding approximately 50% must). A replicated 250 mL sample from each treatment was collected for analysis. Pectolytic enzyme was added (4 g hL^{-1}) to favor settling. SO_2 (50 mg L^{-1}) was added to avoid oxidation. After 24 h, musts were racked and then fermented in 35 L stainless steel tanks. Commercial yeast (Excellence FW, Lamothe-Abiet, Bordeaux, France) was added at a rate of 20 g hL^{-1}. Density and temperature of fermentations were daily monitored. Once alcoholic fermentation finished, wines were racked and sulphited to 35 mg L^{-1} free sulphur dioxide. A natural clarification was carried out at 4 °C for one month. Finally, wines were filtered, bottled and stored.

2.4. Analytical methods

Basic parameters of musts (such as total soluble solids, pH and titratable acidity) and wines (including alcohol content and pH among others) were determined by Fourier transform infrared spectrometry (FTIR) using a WineScan FT120 analyzer (FOSS Electric, Barcelona, Spain) calibrated according to the official methods (OIV, 2009). Analytical determinations in the wines were carried out in triplicate five months after bottling.

2.4.1. Determination of amino acids in musts and wines

The determination of the amino acids present in the Treixadura musts and wines was performed according to Gómez Alonso, Hermosín Gutiérrez, and García Romero (2007) and Garde Cerdán et al. (2009) with slight modifications. This method is based on a reaction of

derivatization in a basic methanolic medium. Determinations were carried out in triplicate.

Briefly, amino acids were determined after reaction of 1.75 mL of borate buffer 1 M (pH = 9), 0.75 mL of methanol, 1 mL of sample (either must or wine) without any pre-treatment, 20 μL of internal standard (L-2-aminoadipic acid, 1 g L^{-1}), and 30 μL of DEEMM in a screw-cap tube over 30 min in an ultrasound bath. Then, the sample was heated at 70 °C for 2 h to degrade the excess of DEEMM and reagent byproducts.

The HPLC analysis was performed using an Agilent 1100 series equipment (Agilent Technologies, Palo Alto, CA, USA). Chromatographic separation was carried out in a Zorbax Eclipse AAA column (C18), particle size 5 μm (150 mm × 4.6 mm; Agilent). A precolumn was also used (Zorbax Eclipse AAA, 12.5 mm × 4.6 mm; Agilent). This column was thermostated at 22 °C.

Elution conditions were as follows: 0.8 mL min^{-1} flow rate, 10% B during 20 min, then elution with linear gradients from 10 to 17% B in 10 min, from 17 to 19% B in 0.01 min, maintained during 0.99 min, from 19 to 19.5% B in 0.01 min, from 19.5 to 23% B in 8.5 min, from 23 to 29.4% B in 20.6 min, from 29.4 to 72% B in 8 min, from 72 to 82% B in 5 min, from 82 to 100% B in 4 min, maintained during 3 min, followed by washing and reconditioning the column. Phase A was 25 mM acetate buffer (pH 5.8) with 0.4 g of sodium azide; phase B was 80:20 (v/v) mixture of acetonitrile and methanol.

The injected volume was 50 μL. A photodiode array detector was used for amino acids and ammonium detection at 280 and 269 nm, respectively. In these conditions, 22 amino acids and the ammonium ion were separated, identified and quantified. These compounds were identified according to the retention times and UV–vis spectral characteristics of the derivatives of the corresponding standards and were quantified using the internal standard method.

The detection (LOD) and quantification (LOQ) limits of the different compounds were calculated as three and ten times, respectively, the standard deviation that provided the signal noise ratio in the lowest concentrations. The LOD values were lower than 0.1 mg L^{-1} except for threonine, where LOD values were lower than 0.3 mg L^{-1} in musts. The LOD values were lower than 0.1 mg L^{-1} in wines.

2.4.2. Determination of volatile compounds

Volatile compounds were determined by gas chromatography (GC). The concentration of major volatile compounds (methanol, higher alcohols, acetaldehyde, ethyl acetate, ethyl lactate, 1-hexanol, acetoine, and 2-phenyl-ethanol) was assessed by direct injection, as proposed by Peinado, Moreno, Muñoz, Medina, and Moreno (2004), in an Agilent 7890A gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a split/splitless injector, electronic flow control (EFC) and a flame ionisation detector. Thus, 50 μL of an internal standard solution (5 g L^{-1} of 4-methyl-2-pentanol in 50% ethanol) were added to 5 mL of wine. An aliquot of 2 μL of this mixture was injected (split 1:30) into a CP-WAX 57CB fused silica capillary column of 50 m × 0.32 mm and 0.2 μm film thickness (Chrompack, Middleburg, The Netherlands). Instrumental conditions were as follows: injector temperature of 275 °C, detector temperature of 300 °C, hydrogen as carrier gas at 3.3 mL min^{-1}, and nitrogen as make-up gas at 30 mL min^{-1}. The flow rates of detector gas hydrogen and air were 40 mL min^{-1} and 400 mL min^{-1}, respectively. The temperature program was as follows: 50 °C for 5 min, increased to 200 °C at a rate of 4 °C min^{-1} and held at 200 °C for 15 min. Total running time was 57.5 min.

Terpenes, C6 alcoholes, volatile fatty acids, ethyl esters of fatty acids and acetates of higher alcohols were extracted (solid phase extraction) according to López Vázquez, Bollaín, Moser, and Orriols (2010), with some modifications: to 100 mL of wine, previously diluted (1:1), the following internal standards were added: for free terpenes and C6 alcohols, 100 μL of 4-decanol (0.144 mg L^{-1}) and, for acids, acetates and esters, 1 mL of 1-heptanol (0.213 g L^{-1}). This mixture was passed through a cartridge Isolute ENV+ SPE (1 g) (Biotage, UK) at a rate of 4–5 mL min^{-1}. Then, this sample was cleaned with 15 mL of distilled water and the free aromatic fraction was eluted with 30 mL of dichloromethane and recovered in a tube. Then, 60 mL of *n*-pentane was added to the eluate, as well as anhydrous sodium sulfate for removing water from the sample. Then, the mixture was passed to a 40 °C bath with a Vigreux column with refrigeration and concentrated to 1.5 mL. Then, the extract was injected in an Agilent 6890 (Palo Alto, CA, USA) gas chromatograph coupled to an Agilent mass spectrometer (MS) model 5973N with a single quadrupole analyzer.

For terpenes and C6 alcohols, extracts (2 μL) were injected (split 1:10) into a polyethyleneglycol HP-Innowax capillary column (30 m × 0.25 μm × 0.25 mm internal diameter; Agilent, Palo Alto, CA, USA) using He (99.999%) as carrier gas at a constant rate of 1 mL min^{-1}. Instrumental conditions were as follows: injector temperature 250 °C. The temperature program was as follows: 35 °C for 2 min, increased to 60 °C at a rate of 30 °C min^{-1}, then at a rate of 2 °C min^{-1} till 160 °C and at a rate of 3 °C min^{-1} till 230 °C, and held at 230 °C for 10 min. Transfer line was kept at 220 °C, whereas ionization source and quadropole temperatures were fixed at 230 °C and 150 °C, respectively. Multiplier voltage was 70 eV. Chromatograms in *full scan* were recorded in the interval between 30 and 300 *m/z* units. Total running time was 97.08 min.

For determining volatile fatty acids, ethyl esters and acetates of higher alcohols, the same column as that for terpenes and C6-alcohols was used but under different conditions. Extracts (3 μL) were injected in split mode (1:7.7), injector temperature was 230 °C, He as carrier gas at a constant rate of 1.3 mL min^{-1}. The temperature program was as follows: 45 °C for 2 min, increased to 230 °C at a rate of 3 °C min^{-1}, and held at 230 °C for 10 min. Transfer line was kept at 220 °C, whereas ionization source and quadropole temperatures were fixed at 230 °C and 150 °C, respectively. Multiplier voltage was 70 eV. Chromatograms in *full scan* were recorded in a range between 25 and 220 *m/z* units. Total running time was 73.67 min.

Volatile compounds were identified by comparing their mass spectra (using the NIST Mass Spectral library) and their retention times with those of commercial pure standards. All determinations were carried out in triplicate.

The odour activity value (OAV) of certain compounds was calculated by dividing their concentration to their odour threshold value (Ferreira, López, & Cacho, 2000; Guth, 1997) for estimating their contribution to the wine sensory profile.

2.5. Sensory evaluation

The sensory evaluation of the wines from each treatment and year was performed by a tasting panel consisting of nine judges, experts in Galician white wines. Experienced tasters are considered to communicate and describe better what they like than consumers or trained panelists (Hopfer & Heymann, 2014). In our case, the panel of judges consisted of five males and four females between 30 and 64 years old. These individuals were enologists and technicians either from EVEGA or from Galician wineries.

A scorecard including 21 descriptors (4 for colour, 10 for aroma, and 7 for palate) was used. The descriptors considered were specifically chosen for Galician white wines (Vilanova, Escudero, Graña, & Cacho, 2013) and were scored from 0 (not present) to 9 (most intense). Furthermore, tasters were asked to provide a mark for the global quality of the wine.

Wine samples (30 mL) coded with random numbers were served in clear tulip-shaped glasses. The tasting sessions were held in different days (April and May from the year after the vintage) at the tasting room of EVEGA. Data were processed using Big Sensory Soft 1.02 (Centro Studi Assagiatori, Brescia, Italy).

Table 1
General parameters of the musts and wines of Treixadura for the three vintages studied (2012, 2013, and 2014). The effects of year, irrigation treatment and their interaction are also shown.

Year	Treatment	Total soluble solids (°Brix)	Titratable acidity (g L^{-1})	pH	Malic acid (g L^{-1})	Tartaric acid (g L^{-1})
Musts						
2012	Rain-fed	22.4 ± 1.5	5.6 ± 0.6	3.42 ± 0.15	2.8 ± 0.2	5.5 ± 0.7
	Irrigated	22.6 ± 1.0	5.7 ± 0.4	3.39 ± 0.08	2.9 ± 0.1	5.4 ± 0.3
2013	Rain-fed	24.9 ± 1.3	5.1 ± 0.4 a	3.64 ± 0.08	2.5 ± 0.4	6.3 ± 0.6
	Irrigated	24.9 ± 1.1	5.8 ± 0.3 b	3.53 ± 0.07	2.8 ± 0.1	6.5 ± 0.2
2014	Rain-fed	22.7 ± 0.4 b	5.8 ± 0.3 a	3.55 ± 0.06	3.5 ± 0.2	6.5 ± 0.4 a
	Irrigated	21.7 ± 0.2 a	6.3 ± 0.1 b	3.47 ± 0.04	3.5 ± 0.3	7.2 ± 0.2 b
Factorial analysis						
Year		**	ns	*	***	***
Treatment		ns	*	ns	ns	ns
Year x Treatment		ns	ns	ns	ns	ns

Year	Treatment	Alcohol (% vol.)	Titratable acidity (g L^{-1})	pH	Malic acid (g L^{-1})	Tartaric acid (g L^{-1})
Wines						
2012	Rain-fed	13.5 ± 0.9	5.2 ± 1.3	3.45 ± 0.39	1.5 ± 1.1	2.0 ± 0.6
	Irrigated	13.6 ± 0.6	5.4 ± 0.6	3.41 ± 0.08	1.4 ± 1.0	2.0 ± 0.1
2013	Rain-fed	14.7 ± 0.7	6.0 ± 0.3	3.40 ± 0.09	2.5 ± 0.4	1.4 ± 0.2
	Irrigated	14.2 ± 1.0	6.5 ± 0.7	3.27 ± 0.14	2.5 ± 0.3	1.8 ± 0.3
2014	Rain-fed	13.6 ± 0.4	6.9 ± 0.6	3.48 ± 0.10	3.1 ± 0.2	1.5 ± 0.3
	Irrigated	13.1 ± 0.2	7.1 ± 0.3	3.38 ± 0.14	2.7 ± 0.1	2.1 ± 0.4
Factorial analysis						
Year		*	*	ns	**	***
Treatment		ns	ns	ns	ns	ns
Year x Treatment		ns	ns	ns	ns	ns

For each year, different letters indicate significant differences between treatments at $p < 0.05$. For the factorial analysis: ns = not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

2.6. Statistical analyses

Two-way analysis of variance (ANOVA) considering year, irrigation treatment and their interaction as factors was used for analyzing data. ANOVA was performed using the SPSS 11.5 for Windows. Mean separation was performed using the Least Significant Difference (LSD) test. Principal Component Analysis (PCA) was carried out using InfoStat Professional 2012 version (www.infostat.com.ar). Those amino acids significantly differing in concentration between treatments were used for the PCA. In addition, this technique allowed us to correlate aromatic descriptors issued from the sensory analysis with those volatile compounds that appeared in concentrations greater than their perception threshold.

3. Results and discussion

3.1. General parameters of musts and wines

Titratable acidity of the musts was significantly increased by irrigation in 2013 and 2014 (Table 1). However, the rest of the parameters determined in musts was not significantly affected by irrigation, except for the reduction in total soluble solids and the increasing in tartaric acid concentration observed in 2014 (Table 1). In contrast, all the must attributes considered in this study were significantly affected by year, except for titratable acidity. No significant interactions between treatment and year were detected (Table 1). The absence of differences in must attributes caused by irrigation are expected due to the mild to moderate water stress conditions achieved in the study area (Trigo Córdoba et al., 2015). The amounts of water applied were similar to those used in cool-humid climates (Balint & Reynolds, 2017).

Therefore, titratable acidity was the most sensitive trait to irrigation, which was scheduled according to weather variables (Trigo Córdoba et al., 2015) and evapotranspiration during the irrigation period was approximately 200 mm, whereas rainfall ranged from 21 to 82 mm over this timespan (Supplementary Table 1). Therefore, water applied only restored half of the theoretical water requirements of Treixadura grapevines in order not to constrain berry quality (Jackson & Lombard, 1993) but significant differences in water status between rain-fed and irrigated vines were detected (Supplementary Table 2 and Trigo Córdoba et al., 2015), which may affect must composition. In fact, in the last year of the study, musts from the rain-fed treatment had higher total soluble solids and lower tartaric acid concentrations than those from the irrigated treatment, as previously observed in other cultivars (Intrigliolo, Pérez, Risco, Yeves, & Castel, 2012). Furthermore, the absence of significant differences between treatments till the last year of experiments might reflect a cumulative effect of irrigation (Junquera, Lissarrague, Jiménez, Linares, & Baeza, 2012). Increases in total soluble solids under water deficit were observed in a red cultivar (Cabernet Sauvignon) but not in a white one (Chardonnay), suggesting different responses of the metabolism of each variety to water deficit (Deluc et al., 2009). In the case reported here, the levels of water stress were low to moderate, depending on the year, and this would have reduced the differences between treatments, as previously observed in white varieties under cool climates (Balint & Reynolds, 2017).

In the case of wines, the irrigation treatment did not exert a significant influence on any of the attributes determined in the current study (Table 1), as observed in irrigation experiments on Chardonnay under cool climate conditions (Balint & Reynolds, 2017). In fact, water stress has shown contradictory effects on wine composition depending on time of water stress within a season and its severity (Intrigliolo et al., 2012; Jackson & Lombard, 1993). Our results indicated that the severity of water stress experienced by Treixadura vines was not enough for significantly altering the main wine attributes. As observed for the musts, year exerted a significant influence on all of the wine attributes except for pH (Table 1). No significant interactions between year and treatment have been detected for any of these attributes.

Table 2
Irrigation effects on the amino acid concentrations (mean ± standard deviation, mg L^{-1}) of musts from Treixadura in AOC Ribeiro. The significances of the year, treatment as well as their interaction are also shown.

Compound	2012		2013		2014		Treatment	Year	Treatment × Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated			
Aspartic acid	55 ± 11	62 ± 12	36 ± 9	36 ± 3	50 ± 7	53 ± 8	ns	ns	ns
Glutamic acid	55 ± 16	53 ± 14	103 ± 19	99 ± 4	100 ± 14	96 ± 12	ns	***	ns
Asparagine	13 ± 4	12 ± 4	6 ± 3	10 ± 2	11 ± 2	13 ± 3	ns	ns	ns
Serine	50 ± 9	51 ± 8	41 ± 10	43 ± 4	43 ± 6	45 ± 5	ns	ns	ns
Glutamine	201 ± 48	258 ± 48	113 ± 48	149 ± 19	268 ± 50	330 ± 96	ns	***	ns
Histidine	31 ± 11	28 ± 9	15 ± 5	15 ± 2	20 ± 4	21 ± 5	ns	*	ns
Glycine	4.0 ± 0.8	3.6 ± 1.3	5.2 ± 0.6	4.9 ± 0.5	5.7 ± 0.6	5.8 ± 0.5	ns	**	ns
Threonine	78 ± 19	63 ± 25	61 ± 14	63 ± 4	67 ± 9	70 ± 9	ns	ns	ns
Arginine	252 ± 71	249 ± 67	222 ± 86	260 ± 28	315 ± 53	368 ± 96	ns	ns	ns
Alanine	127 ± 37	121 ± 20	93 ± 32	102 ± 18	131 ± 17	142 ± 22	ns	ns	ns
γ-Aminobutyric acid (GABA)	68 ± 9	62 ± 10	39 ± 7	32 ± 4	32 ± 1	31 ± 3	ns	***	ns
Proline	**5.1 ± 1.3 a**	**10.4 ± 1.3 b**	4.7 ± 2.6	5.7 ± 0.3	4.3 ± 0.7	4.9 ± 0.8	**	**	*
Tyrosine	12.9 ± 5.5	9.6 ± 3.1	4.8 ± 1.0	4.7 ± 0.5	4.3 ± 1.2	4.0 ± 0.6	ns	***	ns
Ammonium ion	142 ± 6	155 ± 10	217 ± 24	249 ± 14	173 ± 14	179 ± 19	*	***	ns
Valine	20 ± 5	19 ± 4	19 ± 4	17 ± 3	19 ± 3	18 ± 2	ns	ns	ns
Methionine	3.7 ± 1.9	3.1 ± 0.5	2.1 ± 0.4	2.1 ± 0.5	1.3 ± 0.1	1.3 ± 0.1	ns	**	ns
Cysteine	2.1 ± 0.3	2.1 ± 0.2	**2.8 ± 0.2 b**	**1.1 ± 0.1 a**	2.3 ± 0.2	2.3 ± 0.2	***	**	***
Isoleucine	14 ± 7	11 ± 3	10 ± 1	8 ± 1	12 ± 2	11 ± 1	ns	ns	ns
Tryptophan	**18 ± 4 b**	**11 ± 2 a**	13 ± 3	10 ± 1	9 ± 2	8 ± 1	**	*	ns
Leucine	14 ± 4	12 ± 2	10 ± 2	9 ± 1	12 ± 2	12 ± 1	ns	ns	ns
Phenylalanine	27 ± 6	21 ± 4	**24 ± 1 b**	**18 ± 1 a**	23 ± 3	22 ± 2	*	ns	ns
Ornithine	1.9 ± 0.6	2.3 ± 0.9	1.7 ± 0. 8	1.6 ± 0.2	2.0 ± 0.3	2.4 ± 0.8	ns	ns	ns
Lysine	5.9 ± 1.6	5.6 ± 2.0	4.7 ± 1.3	4.6 ± 0.2	5.0 ± 0.4	5.2 ± 0.7	ns	ns	ns
Sum of amino acids	1060 ± 247	1071 ± 194	831 ± 238	897 ± 86	1135 ± 171	1266 ± 264	ns	*	ns

Bold letters indicate significant differences between treatments at $p < 0.05$ for a given amino acid in a given year. The significance of the year, treatment and their interaction is expressed as ns = not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3.2. Amino acids profiles of musts and wines

The amino acid concentrations in Treixadura musts (Table 2) were within previously reported ranges (Bell & Henschke, 2005), except for the low values of proline, which were between 4 and 10 mg L^{-1} when they usually appear in the range of 9–2257 mg L^{-1}. Amino acids concentrations in grapes depend on many factors such as cultivar, maturation stage and management practices (Lee & Schreiner, 2010). In our study, on average for the three years, irrigation tended to increase the concentration of amino acids in musts, which can be tracked in the individual chromatograms (Supplementary Fig. 1).

In this context, some cultivars are considered proline accumulators, whereas others are arginine accumulators (Garde Cerdán et al., 2009). Similarly to Syrah, Petit Verdot, Merlot (Garde Cerdán et al., 2009), Garnacha and Pinot Noir (Bell & Henschke, 2005), Treixadura can be considered arginine accumulator, since this was the most abundant amino acid in its berries, accounting for 30% of the total free amino acids in Treixadura musts (Supplementary Fig. 2). Other amino acids that appeared in relevant concentrations were GABA (11%), threonine (10%), alanine (8%) and glutamic acid (8%).

Irrigation significantly increased proline concentrations in Treixadura musts in 2012, whereas it reduced the concentrations of cysteine in 2013, tryptophan in 2012 and phenylalanine in 2013 (Table 2). Previous studies showed that amino acid metabolism response to water stress in berries depended on the cultivar (Deluc et al., 2009); in the current study, the low differences in amino acid concentrations between treatments might be caused by the mild level of water stress imposed to the vines. Moreover, Deluc et al. (2009) observed that a white cultivar subjected to a level of water stress greater than that imposed in the current study did not show differences in amino acid metabolism, which may indicate that amino acid metabolism in white varieties, such as Treixadura, is more resistant to water deficit than that of red cultivars. The differences observed on individual amino acids within each year might be explained by the particular climate conditions of that year. In our case, the increase in proline concentrations caused by irrigation in 2012 (Table 2) is not expected to have a repercussion in wine aroma since this amino acid is not usually metabolized by yeasts (Bell & Henschke, 2005) and not relevant repercussion on aromatic composition has been achieved (Robinson et al., 2009). In contrast, the reductions in concentrations of cysteine in 2013, tryptophan in 2012 and phenylalanine in 2013 (Table 2) caused by irrigation might have implications on wine composition since some of these amino acids are aroma precursors (Bell & Henschke, 2005), for instance phenylalanine is a precursor of 2-phenylethanol that provides floral notes to wine aroma.

The year exerted a significant influence on the concentrations of ammonium ion and ten amino acids (glutamic acid, glutamine, histidine, glycine, GABA, proline, tyrosine, methionine, cysteine, and tryptophan), which can be explained by the particular climate conditions occurring each year; however, the relative proportion of amino acids was approximately constant over the study period. Ortega Heras et al. (2014) reported that irrigation did not affect the amino acids contents in Verdejo cultivar, except for some individual compounds and concluded that maturation stage and, mainly, climate conditions determined the concentrations of amino acids in the musts; in accordance with our results. In fact, amino acid concentrations in musts for both treatments were lower in 2013 than in 2012 and 2014, which coincided with lower midday stem water potentials, yields and berry weights (Supplementary Table 2; Trigo Córdoba et al., 2015), and also with a certain difference in maturation degree (Table 1). Significant interactions between treatment and year were detected for proline and cysteine concentrations in Treixadura musts (Table 2).

When considering the seven most abundant amino acids in Treixadura musts, PCA was able to separate must samples from each year (Supplementary Fig. 3). The first two principal components (PC) explained 90.2% of the total variance in the dataset: PC1 accounted for 54.3% and PC2 35.9%. Samples from 2012 showed higher concentrations in GABA and aspartic acid, whereas those from 2014 had greater concentrations in arginine and glutamine. The effect of irrigation was not clear and samples from different treatments but belonging to the same year remained rather near in the biplot. Nevertheless, samples from the irrigated treatment in 2014 were separated from those of the

Table 3
Irrigation effects on the concentrations of volatile compounds (mean ± standard deviation) of wines from Treixadura in AOC Ribeiro. The significances of the year, treatment as well as their interaction are also shown.

	2012		2013		2014		Treatment	Year	Treatment × Year
	Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated			
Methanol (mg L^{-1})	26 ± 2	26 ± 1	32 ± 3	33 ± 3	38 ± 2	37 ± 2	ns	***	ns
Ethyl acetate (mg L^{-1})	56 ± 8	74 ± 39	27 ± 2	28 ± 8	34 ± 6	32 ± 4	ns	*	ns
Acetaldehyde (mg L^{-1})	60 ± 13	42 ± 30	33 ± 5	32 ± 2	33 ± 5	32 ± 2	ns	ns	ns
Higher alcohols (mg L^{-1})									
1-propanol	30 ± 9	32 ± 10	38 ± 9	42 ± 3	42 ± 6	40 ± 6	ns	ns	ns
2-methyl-1-propanol	53 ± 9	59 ± 22	35 ± 1	35 ± 2	33 ± 2	30 ± 2	ns	**	ns
2-methyl-1-butanol	44 ± 4	44 ± 2	63 ± 2	58 ± 7	52 ± 6	46 ± 6	ns	***	ns
3-methyl-1-butanol	193 ± 19	181 ± 10	275 ± 10	262 ± 10	226 ± 12	208 ± 14	ns	***	ns
Σ Higher alcohols	321 ± 11	315 ± 25	411 ± 5	397 ± 15	352 ± 21	323 ± 21	ns	***	ns
Other alcohols (mg L^{-1})									
1-hexanol	0.8 ± 0.03	0.8 ± 0.03	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.3 ± 0.04	ns	***	ns
trans-3-hexen-1-ol	0.06 ± 0.003	0.05 ± 0.02	0.05 ± 0.00	0.05 ± 0.00	0.16 ± 0.04	0.15 ± 0.01	ns	***	ns
cis-3-hexen-1-ol	0.42 ± 0.06	0.34 ± 0.15	0.22 ± 0.01	0.26 ± 0.04	0.26 ± 0.04	0.27 ± 0.04	ns	*	ns
Benzyl alcohol	2.89 ± 0.17	3.45 ± 0.53	2.07 ± 0.15	2.08 ± 0.09	2.29 ± 0.24	2.33 ± 0.25	ns	***	ns
2-phenylethanol	26 ± 4	27 ± 6	58 ± 2	51 ± 10	38 ± 5	30 ± 6	ns	***	ns
Other compounds (mg L^{-1})									
Ethyl lactate	31 ± 17	50 ± 34	5 ± 0.2	6 ± 1	5 ± 1	6 ± 1	ns	**	ns
Acetoíne	56 ± 34	40 ± 23	3 ± 1	4 ± 0	3 ± 0	3 ± 0	ns	**	ns
Acetol	70 ± 43	73 ± 34	30 ± 4	31 ± 6	18 ± 3	13 ± 2	ns	**	ns
2,3-butanediol levo	1026 ± 450	878 ± 216	1080 ± 112	1050 ± 125	1119 ± 95	964 ± 58	ns	ns	ns
2,3-butanediol *meso*	416 ± 98	420 ± 88	427 ± 27	427 ± 28	239 ± 21	206 ± 14	ns	***	ns
Methionol	0.22 ± 0.11	0.29 ± 0.02	0.18 ± 0.12	0.22 ± 0.13	0.35 ± 0.10	0.24 ± 0.05	ns	ns	ns
Acetates of higher alcohols (mg L^{-1})									
Isoamyl acetate	0.9 ± 1.1	0.9 ± 0.2	1.2 ± 0.2	1.1 ± 0.4	1.7 ± 0.2	1.5 ± 0.2	ns	ns	ns
Hexyl acetate	0.32 ± 0.20	0.24 ± 0.09	0.21 ± 0.11	0.35 ± 0.11	0.38 ± 0.13	0.32 ± 0.01	ns	ns	ns
2-phenylethyl acetate	0.11 ± 0.11	0.13 ± 0.06	0.52 ± 0.05	0.44 ± 0.05	0.18 ± 0.03	0.15 ± 0.01	ns	***	ns
Σ Acetates of higher alcohols	1.36 ± 0.43	1.25 ± 0.41	1.91 ± 0.49	1.87 ± 0.40	2.21 ± 0.80	1.93 ± 0.71	ns	ns	ns
Esters (mg L^{-1})									
Ethyl butyrate	0.15 ± 0.05	0.12 ± 0.02	< LOD	< LOD	0.10 ± 0.01	0.09 ± 0.02	ns	ns	ns
Ethyl hexanoate	0.52 ± 0.06	0.46 ± 0.10	0.48 ± 0.03	0.46 ± 0.08	0.28 ± 0.05	0.31 ± 0.05	ns	***	ns
Ethyl octanoate	0.83 ± 0.06	0.90 ± 0.18	0.65 ± 0.04	0.64 ± 0.14	0.47 ± 0.15	0.53 ± 0.08	ns	***	ns
Ethyl decanoate	0.09 ± 0.01	0.13 ± 0.03	0.21 ± 0.02	0.23 ± 0.06	0.16 ± 0.04	0.18 ± 0.02	ns	**	ns
Σ Ethyl esters C6-C10	1.59 ± 0.35	1.61 ± 0.37	1.34 ± 0.22	1.33 ± 0.20	1.01 ± 0.16	1.11 ± 0.19	ns	**	ns
Volatile fatty acids (mg L^{-1})									
Isobutyric acid	2.00 ± 0.73	2.19 ± 0.77	2.99 ± 0.11	3.13 ± 0.17	2.30 ± 0.41	2.09 ± 0.31	ns	ns	ns
Butyric acid	1.98 ± 0.29	1.97 ± 0.08	1.54 ± 0.11	1.63 ± 0.13	1.18 ± 0.07	1.21 ± 0.08	ns	***	ns
Isovaleric acid	0.61 ± 0.11	0.65 ± 0.06	2.11 ± 0.07	1.98 ± 0.24	1.48 ± 0.32	1.33 ± 0.26	ns	***	ns
Σ Volatile fatty acids C4-C5	4.59 ± 0.80	4.81 ± 0.83	6.64 ± 0.73	6.74 ± 0.78	4.96 ± 0.58	4.63 ± 0.48	ns	ns	ns
Hexanoic acid	3.57 ± 0.14	3.65 ± 0.29	2.92 ± 0.15	2.97 ± 0.14	2.43 ± 0.37	2.70 ± 0.27	ns	***	ns
Octanoic acid	3.36 ± 0.21	3.55 ± 0.45	2.52 ± 0.14	2.72 ± 0.35	3.09 ± 0.72	3.56 ± 0.59	ns	ns	ns
Decanoic acid	0.51 ± 0.09	0.68 ± 0.27	0.72 ± 0.05	0.78 ± 0.12	1.06 ± 0.31	1.21 ± 0.19	ns	**	ns
Σ Volatile fatty acids C6-C10	7.44 ± 1.71	7.88 ± 1.69	6.16 ± 1.17	6.47 ± 1.20	6.58 ± 1.03	7.47 ± 1.19	ns	ns	ns
Free terpenes (μg L^{-1})									
trans-linalool oxide (furan)†	2.67 ± 0.50	2.86 ± 0.18	5.18 ± 0.44	5.51 ± 1.09	7.26 ± 0.94	7.70 ± 0.21	ns	***	ns
cis-linalool oxide (furan)†	1.76 ± 0.18	1.32 ± 0.30	0.47 ± 0.16	0.59 ± 0.22	0.79 ± 0.34	0.65 ± 0.23	ns	***	ns
trans-linalool oxide (pyran)†	2.92 ± 0.70	1.87 ± 0.47	5.37 ± 1.58	5.88 ± 0.87	5.57 ± 0.07	5.99 ± 1.31	ns	***	ns
cis-linalool oxide (pyran)†	0.48 ± 0.18	0.76 ± 0.22	1.67 ± 0.39	1.47 ± 0.27	0.85 ± 0.04	0.60 ± 0.32	ns	ns	ns
Linalool (L)	3.61 ± 1.07	3.86 ± 1.66	4.30 ± 0.45	4.61 ± 0.74	7.24 ± 0.80	7.35 ± 1.45	ns	***	ns
Hotrienol (3,7-dimethyl-1,5,7-octatriene-3-ol)†	0.71 ± 0.02	0.50 ± 0.14	8.50 ± 3.63	8.93 ± 2.06	7.37 ± 1.49	5.08 ± 1.97	ns	***	ns
α-terpineol (αT)	1.41 ± 0.30	1.20 ± 0.27	4.90 ± 1.15	5.27 ± 0.52	4.05 ± 0.47	4.85 ± 0.23	*	***	ns
Citronellol (C)	< LOD	< LOD	6.10 ± 2.18	6.46 ± 1.24	9.02 ± 1.88	9.34 ± 0.58	ns	***	ns
Nerol (N)	3.50 ± 0.45	3.65 ± 1.21	< LOD	< LOD	0.81 ± 0.35	0.91 ± 0.28	ns	***	ns
Geraniol (G)	5.35 ± 1.26	7.24 ± 0.65	3.72 ± 0.45	3.53 ± 0.13	5.41 ± 0.28	4.99 ± 0.74	*	*	ns
Σ Free terpenes (L + αT + C + N + G)	13.87 ± 1.61	15.95 ± 2.48	19.02 ± 1.02	19.87 ± 1.23	26.53 ± 3.14	27.44 ± 3.16	ns	*	ns
Hodiol I (*trans*-3,7-dimethyl-1,5-octadiene-3,7-diol)†	< LOD	< LOD	1.54 ± 0.46 a	2.40 ± 0.24 b	3.69 ± 0.25	3.71 ± 0.71	ns	***	ns
2,7-dimethyloctane-4,5-diol†	64.58 ± 0.09	50.25 ± 23.48	5.12 ± 2.37	4.77 ± 0.88	143.12 ± 18.78	130.09 ± 15.38	ns	***	ns

Bold letters indicate significant differences between treatments at $p < 0.05$ for a given year. The significance of the year, treatment and their interaction is expressed as ns = not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. LOD means Limit of Detection.
† indicates that the compound is expressed in μg L^{-1} of internal standard.

rain-fed treatment due to the difference in the concentration of glutamine, which was higher for the musts from the irrigation treatment.

In the case of wines, irrigation did not exert a significant influence on any of the concentrations of the determined amino acids (Supplementary Table 3), likely due to the fermentation process minimizing differences between treatments. Moreover, the level of water

stress experienced by Treixadura vines was low to moderate, due to the climate conditions of the study period, and this caused that must composition was similar between treatments and, therefore, no significant differences were expected for wines, as previously reported for Chardonnay (Balint & Reynolds, 2017). In contrast, the year significantly altered the concentrations of 12 amino acids: aspartic acid, glutamic acid, serine, threonine, arginine, tyrosine, valine, methionine, isoleucine, tryptophan, leucine and lysine. No significant interactions between year and treatment were detected. As observed for the musts, amino acids concentrations in wines were lower in 2013 than in the other two studied years. Arginine and glutamine were the amino acids that yeast used preferently during fermentation, whereas glycine, proline and lysine were found in wines in greater concentrations than in the corresponding musts due to yeast autolysis (Martínez-Rodríguez, Carrascosa, & Polo, 2001). Furthermore, other authors (Deluc et al., 2009; Grimplet et al., 2009) found differences in amino acid metabolism due to water stress in a red cultivar, but they did not detect differences for a white cultivar; in accordance with our results. This absence of differences among treatments may have been caused by the low level of water stress experienced by Treixadura vines under the current experimental conditions.

3.3. Volatile composition of Treixadura wines

A total of 43 compounds were quantified by GC in Treixadura wines, including higher alcohols, acetates, esters, volatile fatty acids and terpenes. Irrigation only affected the concentrations of two compounds in a significant way: α-terpineol and geraniol; likely due to the mild water stress experienced by the vines during the study period. Wines from the irrigation treatment showed greater concentrations of α-terpineol when compared to those from the rain-fed treatment, whereas the opposite was observed for geraniol (Table 3). In contrast, the concentrations of most of the volatile compounds were significantly altered by year. However, no significant interactions between treatment and year were detected. Recently, Talaverano et al. (2017) found higher concentrations of alcohols, C6 compounds and phenol volatiles in Tempranillo wines from the rain-fed treatment than in those from the deficit irrigated treatments, whereas the highest concentrations of ethyl esters were detected in wines from the irrigated treatments. These findings seem to contrast with our results maybe because of the different variety and climate conditions, which caused a higher level of water stress than that experienced by Treixadura vines in our study.

The concentrations of methanol and ethyl acetate were slightly lower in the wines of the current study than those reported for wines from the same variety (Blanco et al., 2012), but those of acetaldehyde were greater, especially in 2012. High contents of acetaldehyde in white wines can indicate oxidation (Swiegers et al., 2005) and might negatively affect the sensory perception of wines, as observed for other Galician varieties such as 'Albariño' and 'Godello' (Blanco, Mirás-Avalos, Pereira, & Orriols, 2013).

In this study, higher alcohols in wines from both treatments appeared at concentrations greater than the threshold considered to contribute negatively to wine complexity, 300 mg L^{-1} (Hirst & Ritcher, 2016), and were much higher than those previously reported for this variety (Blanco et al., 2012), especially the concentration of 3-methyl-1-butanol. These results might have been caused by the different yeast strains used in previous research compared to the one used in the current study.

In contrast, other alcohols imparting positive nuances, such as 2-phenylethanol, appeared in greater concentrations than those previously reported for Treixadura wines (Blanco et al., 2012), likely due to the different yeasts used by those authors. This compound would

Table 4
Irrigation effects on the odour activity values (mean ± standard deviation) of wines from Treixadura in AOC Ribeiro. Odour thresholds and descriptors for each compound are displayed. The significances of the year, treatment as well as their interaction are also shown.

	Odour threshold (μg L^{-1})	Odour descriptor (μg L^{-1})	2012		2013		2014		Treatment	Year	Treatment × Year
			Rain-fed	Irrigated	Rain-fed	Irrigated	Rain-fed	Irrigated			
Ethyl acetate	7500	Pineapple	8 ± 1	10 ± 5	4 ± 1	4 ± 1	5 ± 0	4 ± 0	ns	*	ns
Acetaldehyde	500	Fruity	120 ± 27	84 ± 66	66 ± 12	64 ± 4	66 ± 10	64 ± 4	ns	ns	ns
Higher alcohols											
1-propanol	750	Alcohol	40 ± 12	43 ± 15	51 ± 14	56 ± 4	56 ± 9	53 ± 9	ns	ns	ns
2-methyl-1-propanol	40000	Alcohol	1 ± 0	2 ± 1	< 1	< 1	< 1	< 1	ns	*	ns
3-methyl-1-butanol	30000	Alcohol	6 ± 1	6 ± 0	9 ± 0	9 ± 0	8 ± 0	7 ± 1	ns	***	ns
Other alcohols											
cis-3-hexen-1-ol	400	Grass	1 ± 0	< 1	< 1	< 1	< 1	< 1	ns	ns	ns
Benzyl alcohol	620	Blackberry	5 ± 0	6 ± 1	3 ± 0	3 ± 0	4 ± 0	4 ± 0	ns	***	ns
2-phenylethanol	14000	Rose	2 ± 1	2 ± 0	4 ± 0	4 ± 1	3 ± 0	2 ± 0	ns	***	ns
Other compounds											
Acetoine	10000	Butter, almond	6 ± 3	4 ± 2	< 1	< 1	< 1	< 1	ns	*	ns
Acetates of higher alcohols											
Isoamyl acetate	30	Banana	31 ± 35	29 ± 7	39 ± 7	36 ± 15	55 ± 5	49 ± 6	ns	ns	ns
2-phenyl acetate	250	Rose, honey	< 1	< 1	2 ± 0	2 ± 0	< 1	< 1	ns	ns	ns
Esters											
Ethyl butyrate	20	Fruity	8 ± 3	6 ± 1	< 1	< 1	< 1	< 1	ns	*	ns
Ethyl hexanoate	14	Fruity	37 ± 4	33 ± 7	34 ± 2	33 ± 6	20 ± 4	22 ± 4	ns	***	ns
Ethyl octanoate	5	Fruity	166 ± 12	180 ± 35	130 ± 8	128 ± 27	94 ± 30	106 ± 16	ns	***	ns
Ethyl decanoate	200	Grape	< 1	< 1	1 ± 0	1 ± 0	< 1	< 1	ns	ns	ns
Volatile fatty acids											
Isobutyric acid	2300	Cheese	< 1	< 1	1 ± 0	1 ± 0	< 1	< 1	ns	ns	ns
Butyric acid	173	Cheese	11 ± 2	11 ± 0	9 ± 1	9 ± 1	7 ± 0	7 ± 0	ns	***	ns
Isovaleric acid	33	Cheese	19 ± 3	20 ± 2	64 ± 2	60 ± 7	45 ± 10	40 ± 8	ns	**	ns
Hexanoic acid	3000	Cheese	119 ± 5	122 ± 10	97 ± 5	99 ± 5	81 ± 12	90 ± 9	ns	***	ns
Octanoic acid	500	Rancid	7 ± 0	7 ± 1	5 ± 0	5 ± 1	6 ± 1	7 ± 1	ns	*	ns
Decanoic acid	1000	Rancid	< 1	< 1	< 1	< 1	1 ± 0	1 ± 0	ns	ns	ns

The significance of the year, treatment and their interaction is expressed as ns = not significant; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. Values have been rounded in order to avoid decimals. Only the compounds with odour activity values greater than 1 are shown. Odour thresholds have been taken from Guth (1997) and Ferreira et al. (2000).

contribute positively to wine aroma by providing notes to roses (Aznar, López, Cacho, & Ferreira, 2001). In the case of 2-phenylethanol, year exerted a highly significant influence on its concentration, and the wines from 2013 showed greater concentrations of this compound, which appeared in slightly higher concentrations in those wines coming from the rain-fed treatment (Table 3), likely due to a greater concentration on phenylalanine, which is a precursor, in the musts from the rain-fed treatment (Table 2).

Other compounds such as ethyl lactate, acetoine and acetol, appeared in greater concentrations in wines from 2012 when compared with those from 2013 and 2014. Since ethyl lactate is related to malolactic fermentation, it seems that an spontaneous malolactic fermentation occurred in 2012 wines.

The concentration of acetates did not differ between treatments and among years, except for that of 2-phenylethyl acetate (Table 3). Isoamyl acetate was the most important compound of this group, and it was found in concentrations similar to those previously reported for wines of this variety (Blanco et al., 2012; Falqué et al., 2002). Since acetates provide floral notes to wine aroma, especially 2-phenylethyl acetate (Gómez Míguez, Cacho, Ferreira, Vicario, & Heredia, 2007), wines from 2013 should have a more floral aromatic profile than those from the other studied years.

Ethyl esters constitute a relevant group of chemicals that impart fruity notes, affecting wine aroma. Since they are produced by yeasts during fermentation as secondary products of sugar metabolism (Lambrechts & Pretorius, 2000), irrigation is not supposed to alter significantly their concentrations, as occurred in the current study (Table 3). However, year exerted a significant effect on them, except for ethyl butyrate. The concentrations of these compounds were higher in 2012 than in the rest of the years studied, but these concentrations were very similar between treatments. Furthermore, ethyl esters in Treixadura wines were present at concentrations within the ranges reported in the literature (Sumby, Grbin, & Jiranek, 2010) but at lower levels than in other Galician varieties such as Albariño and Godello (Blanco et al., 2013).

Similarly to esters, no variations as a function of treatment were observed for the total concentration in volatile fatty acids (Table 3). However, year exerted a significant effect on the concentrations of butyric, isovaleric, hexanoic and decanoic acids. The highest concentrations of these compounds were found in wines from 2012, except for isovaleric and decanoic acids, which were observed in 2013 and 2014, respectively. These molecules may contribute negatively (rancid, cheese notes) to wines (Lambrechts & Pretorius, 2000; Swiegers et al., 2005) but they are originated from metabolism of fatty acids by yeasts and bacteria and that is the reason why irrigation did not exert a significant effect on them.

Terpenes are important contributors to the aroma of white wines made from aromatic non-Muscat varieties and correlations between floral sensory attributes and high levels of α-terpineol and free linalool have been documented (Robinson et al., 2014). In our case, free terpenes did not show significant differences between treatments except for α-terpineol (that provides green aromas such as pine or coniferous) that was found in greater concentrations in the wines from the irrigation treatment, and geraniol (notes to rose, geranium), which appeared in greater concentrations in wines from the rain-fed treatment (Table 3). However, these compounds appeared at concentrations lower than their perception thresholds: 250 μg L^{-1} for α-terpineol (Ferreira et al., 2000) and 30 μg L^{-1} for geraniol (Guth, 1997); therefore, their relevance for wine aroma would be minimal. Moreover, hodiol I was present at significantly higher concentrations in wines from the irrigation treatment in 2013. However, the magnitude of these values is low for all this family of compounds. Under acidic conditions, a given terpene can be transformed into another one (for instance, geraniol can be transformed to α-terpineol) or even to less aroma intensive compounds (Ribéreau-Gayon, Boidron, & Terrier, 1975).

When considering OAV as an index of the relevance of a given compound to wine aroma, in our case, only 21 compounds were present at higher concentrations than their corresponding perception threshold (Table 4), although not all the studied years. Among these compounds, the ones with the greatest OAV were ethyl octanoate, hexanoic acid and acetaldehyde. Moreover, no significant differences were observed between treatments for any of these compounds due to a high variability within the replicated wines from each treatment. In contrast, year exerted a significant influence on the OAV of 13 of these compounds. No significant interactions were detected between irrigation and year.

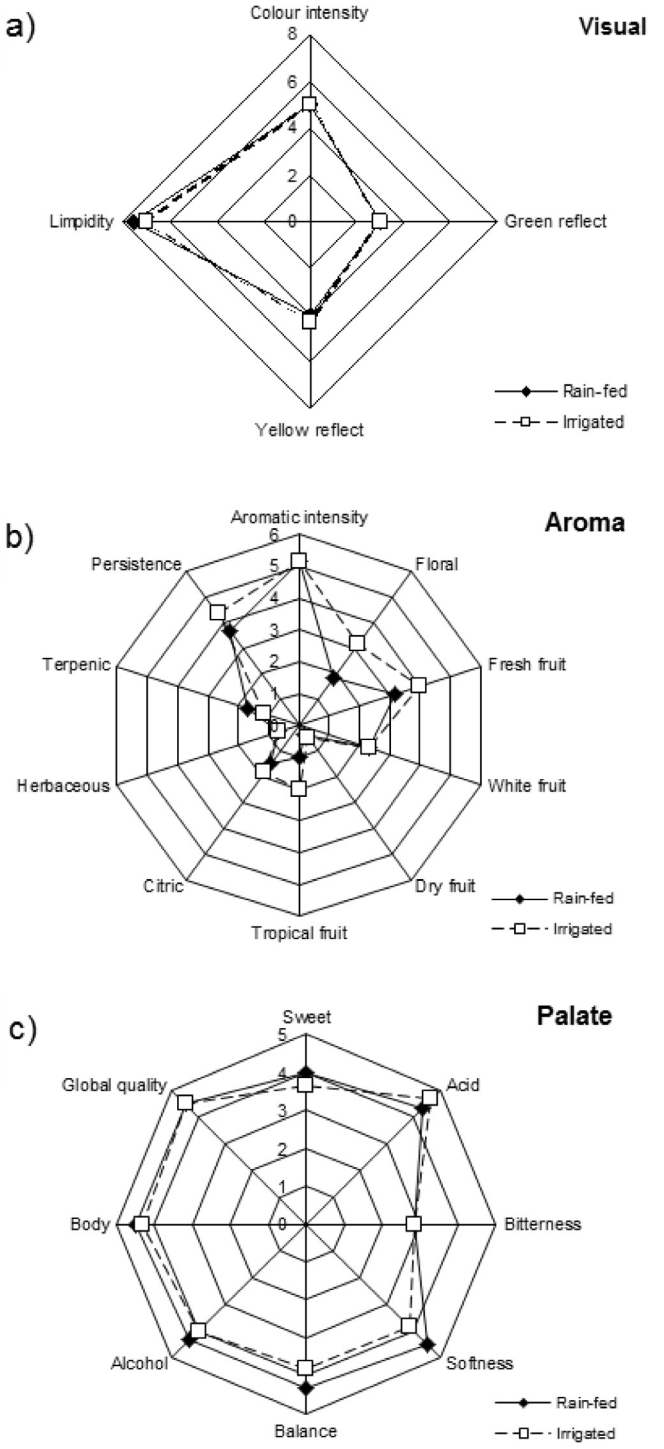

Fig. 1. Sensory profile of Treixadura wines (average values for 2012–2014) as a function of rain-fed and irrigation conditions: a) visual; b) aroma and c) palate descriptors.

Therefore, the ethyl octanoate has been the compound with the greatest impact on Treixadura wines, being responsible for fruity notes. Acetaldehyde is the major aldehyde in wines and is associated with 'fruity' aromas; whereas hexanoic acid may affect negatively to wine aroma by providing cheese, fatty and rancid notes. These two compounds are always present in wines and they constitute the "aroma basis" (Escudero et al., 2004), together with alcohols (isobutanol, isoamyl alcohols, 2-phenylethanol) and their acetates, fatty acids (acetic, butyric, hexanoic, octanoic, isobutyric and 3-methylbutyric) and their ethyl esters, methionol, acetaldehyde, diacetyl and β-damascenone.

3.4. Sensory profiles of Treixadura wines

Fig. 1 shows the sensory profile for Treixadura wines from the rain-fed and irrigation treatments, as averaged for the three studied years. The visual descriptors did not differ between treatments. However, wines from the irrigated treatment received higher marks for aroma descriptors, such as floral and tropical fruit, than those wines from the rain-fed treatment. In contrast, palate descriptors revealed a greater preference of the tasters for wines from the rain-fed treatment, due to higher marks in softness and balance (Fig. 1). Interestingly, over the three years of study, four descriptors were common for describing wine global quality: softness, balance, alcohol and body. Taken each year individually, wines from the different treatments were similarly punctuated, except for 2014 when rain-fed wines received higher marks for overall quality (Supplementary Fig. 4).

In those wines obtained from the irrigated treatment, the floral and fruity attributes have taken precedence in wine aroma may be due to higher concentrations of compounds that provide those notes, such as terpenes, benzyl alcohol and ethyl esters. The reason for wines from the rain-fed treatment had greater ratings on palate descriptors may be attributed to their lower titratable acidity and greater alcohol degree.

Previous reports on red varieties have provided similar results for the sensory evaluation of Cabernet Sauvignon (Chapman, Roby, Ebeler, Guinard, & Matthews, 2005); whereas in white varieties such as Chardonnay (Balint & Reynolds, 2017) the sensory evaluation of wines from different irrigation treatments also indicated that those wines from the irrigation treatments were more acidic in the mouth but showed high aroma complexity at the olfactory level.

3.5. Relationships between volatile composition and sensory profiles

The PCA considering those volatiles with OAV greater than one and the aroma descriptors revealed a clear separation of the wines produced each year, independently from the treatment where they came from (Fig. 2). The first two principal components accounted for 78.2% of the total variance in our dataset: PC1 explained 55% of this variance and PC2 explained 23.2%. Wines from 2012 were located in the positive side of PC1 due to their higher concentrations in acetaldehyde and hexanoic acid, which were related to higher marks of aroma intensity in the tasting sessions. In contrast, wines from 2014 were located in the negative side of PC1 and were related to higher concentrations of isoamyl acetate, which reflected greater punctuations in white fruit, terpenic and persistence descriptors. Interestingly, wines from 2013 were located in the negative side of both PC1 and PC2 due to their greater concentrations in 2-phenylethanol, 3-methyl-1-butanol and ethyl octanoate, but they were not related to any sensory descriptor.

4. Conclusions

The current study provided a clear picture of the effects that irrigation scheduled according to vine water needs may have on Treixadura must and wine compositions under the climate conditions of Galicia. Titratable acidity of the musts increased with irrigation. The concentrations of proline, cysteine, tryptophan and phenylalanine in musts were significantly affected by irrigation. However, volatile composition of wines was not significantly altered by irrigation except for the concentrations of α-terpineol and geraniol. A correlation between volatile compounds with concentrations higher than their perception thresholds and aroma descriptors has been found. In conclusion, in spite of the fact that irrigation affected musts and wine composition in specific cases, climate conditions have been determinant in the results obtained in this study.

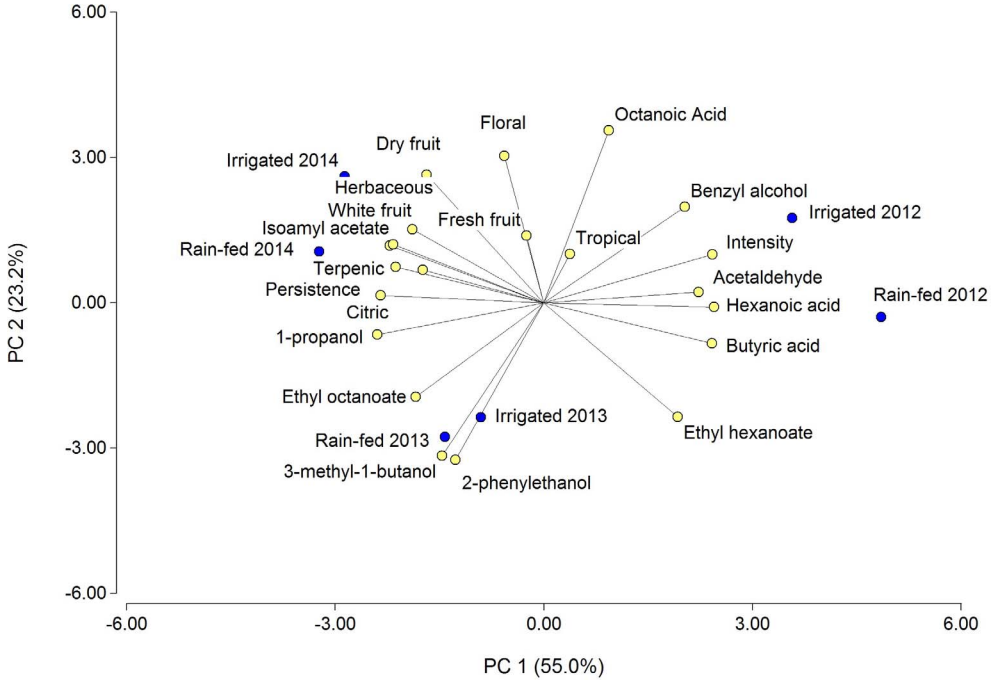

Fig. 2. Principal component analysis (PCA) of Treixadura wines: Biplot for the first two components (PC) for volatile compounds with odour activity values greater than one and the sensory descriptors related to wine aroma.

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Conflict of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.08.013.

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